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(54) Title: TAXOL-BASED COMPOSITIONS WITH ENHANCED BIOACTIVITY

(57) Abstract

Taxol-based compositions are disclosed based on the formation of 7 carbamates. In certain aspects of the invention, the compositions include polyethylene glycol and have prolonged circulating lives in mammals, are highly water soluble and substantially non-antigenic. Methods of preparation and treatment using the compositions are also disclosed.

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TAXOL-BASED COMPOSITIONS WITH ENHANCED BIOACTIVITY

BACKGROUND OF THE INVENTION

This is a continuation-in-part of co-pending U.S. Patent Application Serial Number 07/934,131, filed August 21, 1992, the contents of which are hereby incorporated by reference.

The present invention relates to compositions having an anti-microtubule activity. In particular, the invention relates to modified taxol-based compositions, which, in certain cases, demonstrate prolonged anti-neoplastic activity.

Recently, taxol has been investigated as a possible anti-cancer agent. Taxol is a plant product derived in minute quantities from the needles and bark of the western pacific yew, Taxus brevifolia. In chemotherapy, taxol is known as an anti-microtubule agent and is thought to inhibit cell mitosis through the enhancement of the rate of microtubular assembly and prevention of microtubular depolymerization. Numerous studies performed to date indicate that taxol has a wide spectrum of activity against several malignancies. To date, the use of taxol, however, has been severely limited by, among other things, its short supply, poor water solubility and immunogenicity.

The pacific yew is a rare, slow-growing tree which is not typically cultivated. In addition, the anti-neoplastic portions of the tree are very minute. Extraction of these portions is complicated and costly. One solution to the problem of short supply has been suggested in U.S. Patent No. 5,019,504 which discloses an artificial media capable of producing the certain

desirable alkaloids found in the tree. Alternatively, synthetic derivatives such as taxotere and taxol intermediates have also been reported. See, for example, U.S. Patent No. 5,015,744 which discloses the preparation of taxol intermediates using oxazinones.

The poor water solubility of taxol has also hindered the development of pharmaceutical formulations. U.S. Patent Nos. 5,059,699, 4,960,790 and 4,942,184, contain suggestions for improving the water solubility of taxol. The '699 disclosure is directed to sulfonated 2' acryloytaxol and sulfonated 2' O-acyl acid taxol derivatives. The '790 reference discloses formation of taxol derivatives containing amino acid residues. The '184 patent discloses succinyltaxols. The increased solubility provided by these methods, however, tends to be short-lived in vivo. The ester derivative solubilizing groups used to modify the molecules are quickly hydrolyzed, regenerating the poorly soluble, antigenic parent moieties.

Hypersensitivity reactions from taxol administration are known. See, for example, J. Clin Oncol 8:1263-1268 (1990). Indeed, since taxol is extracted from a natural plant source, hypersensitivity reactions in a portion of the population are expected. Moreover, certain non-aqueous vehicles which have been suggested to deliver taxol in vivo have also been implicated in causing hypersensitivity reactions in mammals.

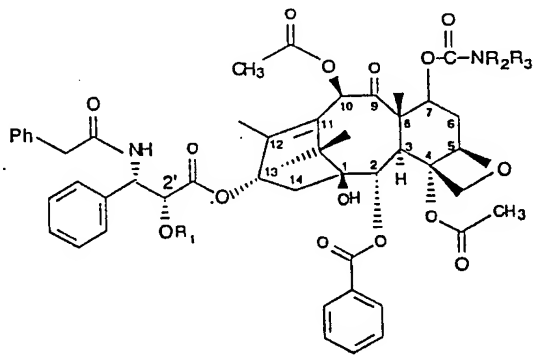
Finally, although taxol holds much promise as an anti-neoplastic agent, certain neoplasms have demonstrated resistance against it's activity. It would be highly desirable to provide novel anti-microtubule compositions with effectiveness against a wider range of

In view of the foregoing, it is an object of the present invention to address the shortcomings set forth above.

SUMMARY OF THE INVENTION

The present invention includes biologically active compositions having a taxol-like activity. The compositions have the structure:

(I)



wherein:

R_1 = H or -CO-X, wherein X = alkyl, haloalkyl, substituted alkyl or aryl;

R_2 = one of H, CH_3, alkyl, cycloalkyl, aryl, aralkyl, aryl$(CH_2)_nY$ or $(CH_2)_nY$, wherein n = 1-12 and Y = OH, NH_2, CHO or $COOR_4$ where R_4 = alkyl, substituted alkyl, phenyl or substituted phenyl, or an α-substituted polyalkylene oxide derivative; and

R_3 = one of H, CH_3, alkyl, cycloalkyl, aryl, aralkyl or an α-substituted polyalkylene oxide derivative.

In those aspects of the invention where one or both of R_2 and R_3 is (are) α-substituted polyalkylene oxide derivatives, the derivatives are in a functionalized or

activated form. One preferred activated polymer is monomethoxy-polyethylene glycol or MPEG.

The present invention also includes methods of making the compositions described herein. The methods include reacting suitable taxol-based moieties with an activating reagent under conditions sufficient to form the biologically active composition. Some suitable reagents include:

R_2NCO where R_2 is one of CH_3, alkyl, cycloalkyl, aryl, aralkyl, $(CH_2)_nY$, wherein n = 1-12 and Y = OH, NH_2, CHO or $COOR_4$ where R_4 = alkyl, substituted alkyl, phenyl or substituted phenyl, or an α-substituted polyalkylene oxide derivative; and

R_2R_3NCOCl where R_2 and R_3 are independently one of CH_3, alkyl, cycloalkyl, aryl, aralkyl or an α-substituted polyalkylene oxide derivative;

Methods of treatment using the compositions described herein are also disclosed.

DETAILED DESCRIPTION OF THE INVENTION

The compositions of the present invention are based on the premise that taxol and taxol-like molecules can be modified in the 7 position to provide improved variations of the naturally occurring alkaloids. The compositions are further described as having an anti-microtubule activity in vivo, especially as such action pertains to oncologic or anti-neoplastic activity as such activity is understood by those of ordinary skill in the art. For example, the compositions in some instances will demonstrate the ability to preferentially bind to and stabilize microtubules, thus interrupting cell mitosis.

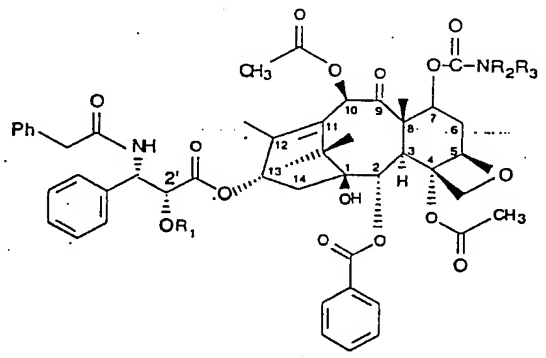
While applicants are not bound by theory, it is believed that other anti-microtubule or oncolytic effects may also be observed in vivo with one or more of the compositions described herein.

For purposes of the present invention, the taxol component of the novel compositions can be selected from a wide variety of materials in addition to taxol per se harvested from naturally-occurring pacific yews and available from, for example, Calbiochem Corp. of San Diego, CA or ESCAgenetics Corp. of San Carlos, CA. For ease of description in the present invention, "taxol" will be understood to include all naturally occurring alkaloids as well as all synthetic and related moieties. A non-limiting list of suitable taxol-based moieties are described in Biochem. Biophys. Res. Comm. 124, 329 (1984); J. Med. Chem. 35, 145 (1992); J. of Nat'l Prod. 51, 298 (1988); J. Med. Chem. 32, 788 (1989) and S.B. Horowitz, et al Annals NY Acad. of Sci. 466, 733 (1986).

The artisan can also synthesize anti-micro tubule moieties such as taxotere based on need. Furthermore, 2' taxol esters can also be used. Since esters hydrolyze in the acidic environment of cancer cells, 2' taxol esters are useful as a prodrug. See J. Med. Chem. 32, 788 (1989). While such pro-drug modifications are desirable in certain situations, it has been surprisingly found that the modifications described herein which are realized by conversion of the 7 OH to relatively stable carbamates provides novel compositions which are chemotherapeutically active. Moreover, 2' taxol esters can be modified in the 7 position if desired, to provide compositions which display both the prodrug and 7-carbamate features.

In addition to the preferred taxol-moieties described above, other moieties having anti-microtubule activity in mammals such as vinca alkaloids are modifiable with the polymers described herein. A review of anti-microtubule agents is set forth in Pharmac. Ther. **52**, pp 35-84 (1991), the text of which is hereby incorporated by reference. The preferred taxol-based compositions of the present invention have the formula:

(I)



wherein:

R_1 = H or -CO-X, wherein X = alkyl, haloalkyl, substituted alkyl or aryl; and R_2 and R_3 are independently one of H, CH_3, alkyl, cycloalkyl, aryl, aralkyl or an α-substituted polyalkylene oxide derivative.

In one aspect of the invention, one of R_2 and R_3 is an α-substituted polyalkylene oxide derivative. Alternatively, both R_2 and R_3 are α-substituted polyalkylene oxide derivatives. In each of these embodiments, the compositions have one or more of the following attributes:

1. improved solubility;
2. reduced antigenicity / immunogenicity;

3. prolonged circulating life; and

4. increased oncolytic activity against resistant cell lines when compared to the naturally occurring alkaloid or taxol-based derivatives not modified in accordance with this embodiment.

The attachment of the polymeric materials to taxol is preferably via a covalent linkage, and most preferably via a carbamate (urethane) linkage. Such linkages are preferred due to their stability, especially in aqueous based systems. It is to be understood, however, that linkage can be achieved via any suitable linking group containing at least one atom capable of joining the taxol moiety covalently to the polymeric material while substantially maintaining the activity of the taxol-containing substance. Alternative linking groups also include ethers. While it is preferred that at least one of R_2, and R_3 is covalently attached to the taxol moiety, one or both may also be attached using reversible and/or ionic or non-covalent chemistries.

Oftentimes, the polymers are activated in order to effect such linkages. By activation, it is understood by those of ordinary skill in the art that the polymer is functionalized to include the desired reactive group. See, for example, U.S. Patent Nos. 4,179,337 and 5,122,614, wherein the hydroxyl end-groups of polyalkylene glycols, are converted and activated into reactive functional groups to modify proteins and/or enzymes. The disclosure of each of the '337 and '614 patents is hereby incorporated by reference. These references, however, are directed to modifying enzymes and/or proteins via epsilon amino acid lysines. The differences in structure, function and effect between

these materials and taxol-based moieties are so substantial that the references are of little predictive value for the purposes of the present invention.

Alternative activated polymers include isocyanates as set forth in the parent U.S. patent application Serial Number 07/934,131, filed August 21, 1992 or the hydrazines set forth in commonly assigned PCT patent application bearing Publication No. WO92/16555.

Polyethylene glycols are particularly preferred polymeric materials. Although polyethylene glycols come in a variety of molecular weights, molecular weight ranges of from about 200 to about 10,000, are usually selected for the purposes of the present invention. Molecular weights of from about 2,000 to about 7,500 are preferred while molecular weights of about 5,000 are particularly preferred. Within this aspect of the invention, it is to be understood that alkyl-capped polyethylene oxides such as methoxypolyethylene glycols (MPEG) and bis-polyethylene oxides are also contemplated.

The polymeric substances included herein are also preferably water-soluble at room temperature. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers maintained.

As an alternative to PAO based polymers, effectively non-antigenic materials such as dextran, polyvinyl pyrrolidone, polyacrylamides polyvinyl alcohols,

carbohydrate-based polymers and the like. Those of ordinary skill and the art will realize that the foregoing list is merely illustrative and that all such polymeric materials having the qualities described herein are contemplated.

The polymeric materials are also effectively non-antigenic in mammals. For purposes of the present invention, "effectively non-antigenic" means all polymeric materials understood in the art as being nontoxic and not eliciting an appreciable immunogenic response in mammals.

In this aspect of the invention, the taxol-polymer conjugates retain at least a substantial portion of the anti-microtubule activity of the taxol moiety prior to conjugation. In most cases, however, the modified compositions demonstrate equipotent and even synergistic activity against certain neoplastic cells. See Examples below. For purposes of the present invention, the terms "substantial portion of the activity" means that at least therapeutic effectiveness is maintained. While the conjugate may have less activity than the unmodified taxol-moiety in certain situations, the therapeutic effectiveness is nonetheless maintained. In any event, the advantageous properties of high aqueous solubility, substantially longer circulating life, and reduced antigenicity, either alone or in combination outweigh any decrease in activity in those situations where decreased but nonetheless therapeutically effective compositions result.

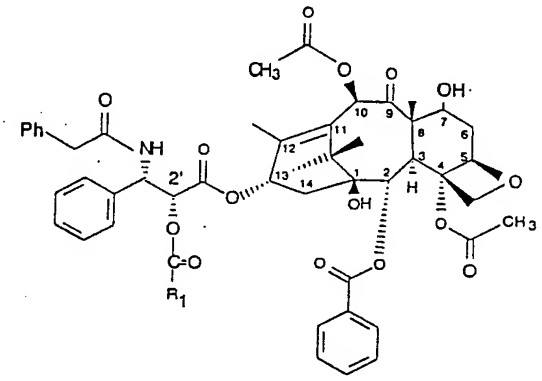
In those aspects of the invention where R_1 and/or R_2 are not α-substituted polyalkylene oxide derivatives,

that is, R_1 and/or R_2 is hydrogen, an alkyl, cycloalkyl, aryl, aralkyl, other 7 carbamate taxols are formed. While these compositions have utility in their own right, they can be further functionalized to include other moieties which would enhance the anti-microtubule composition. For example, the artisan can enhance aqueous solubility by adding moieties such as carboxylic, sulfonic, phosphonic acids or amines or other salt formers. Furthermore, when R_2 = aryl$(CH_2)_nY$ or $(CH_2)_nY$, wherein n = 1-12 and Y = OH, NH_2, CHO or $COOR_4$ where R_4 = alkyl, substituted alkyl, phenyl or substituted phenyl, the artisan is provided with an intermediary composition which can be further modified selectively in the 7 position. Those skilled in the art will recognize that when Y = OH, NH_2 or CHO, these functional groups are added initially in a protected form such as a tetrahydropyranyl ether of the alcohol, carbobenzyloxy for the amine and dialkylacetal for the aldehydes. In this embodiment, an α-substituted polyalkylene oxide derivative can attached via an alkyl spacer to produce an active, highly water soluble anti-microtubule or cytotoxic composition.

In still another aspect of the invention, the compositions are further modified to include a targeting moiety that enhances accumulation of the taxol-based moiety in a desired location such as a tumor. In this regard, suitable targeting moieties include peptide sequences, mono- or polyclonal antibodies, single chain antigens (sFv's), fusion proteins or the like. For example, a DNA binding peptide can be attached to the compositions via a properly functionalized R_2 and/or R_3 under conditions known to those of ordinary skill in the art.

The taxol-based compositions are prepared by reacting a taxol-based moiety depicted below as (II) or one of those described above with an activating reagent out under conditions which are sufficient to effect the desired 7 position modification yet maintain at least a portion of the therapeutic effect of the moiety.

(II)



R_1 = alkyl, haloalkyl, substituted alkyl or aryl.

Usually, in order to effect such 7 position modification, the 2′ position must be blocked. The temporary formation of an acetate with the 2′ OH has also been demonstrated to suffice. Blockage may be carried out in the manner described in Biochem. Biophys. Res. Comm 124, 329 (1984) or J. Med. Chem. 35, 145 (1992).

The activating reagents useful in the formation of the 7 carbamates are generally described as including:

1) R_2NCO where R_2 is one of CH_3, alkyl, cycloalkyl, aryl, aralkyl, aryl$(CH_2)_nY$ or $(CH_2)_nY$, wherein n = 1-12 and Y = OH, NH_2, CHO or $COOR_4$ where R_4 = alkyl, substituted alkyl, phenyl or substituted phenyl, or an α-substituted polyalkylene oxide derivative;

2) R_2NH_2 where R_2 is one of H, CH_3, alkyl, cycloalkyl, aryl, aralkyl or an α-substituted polyalkylene oxide derivative;

3) R_2R_3NH where R_2 and R_3 are independently one of CH_3, alkyl, cycloalkyl, aryl, aralkyl or an α-substituted polyalkylene oxide derivative; or

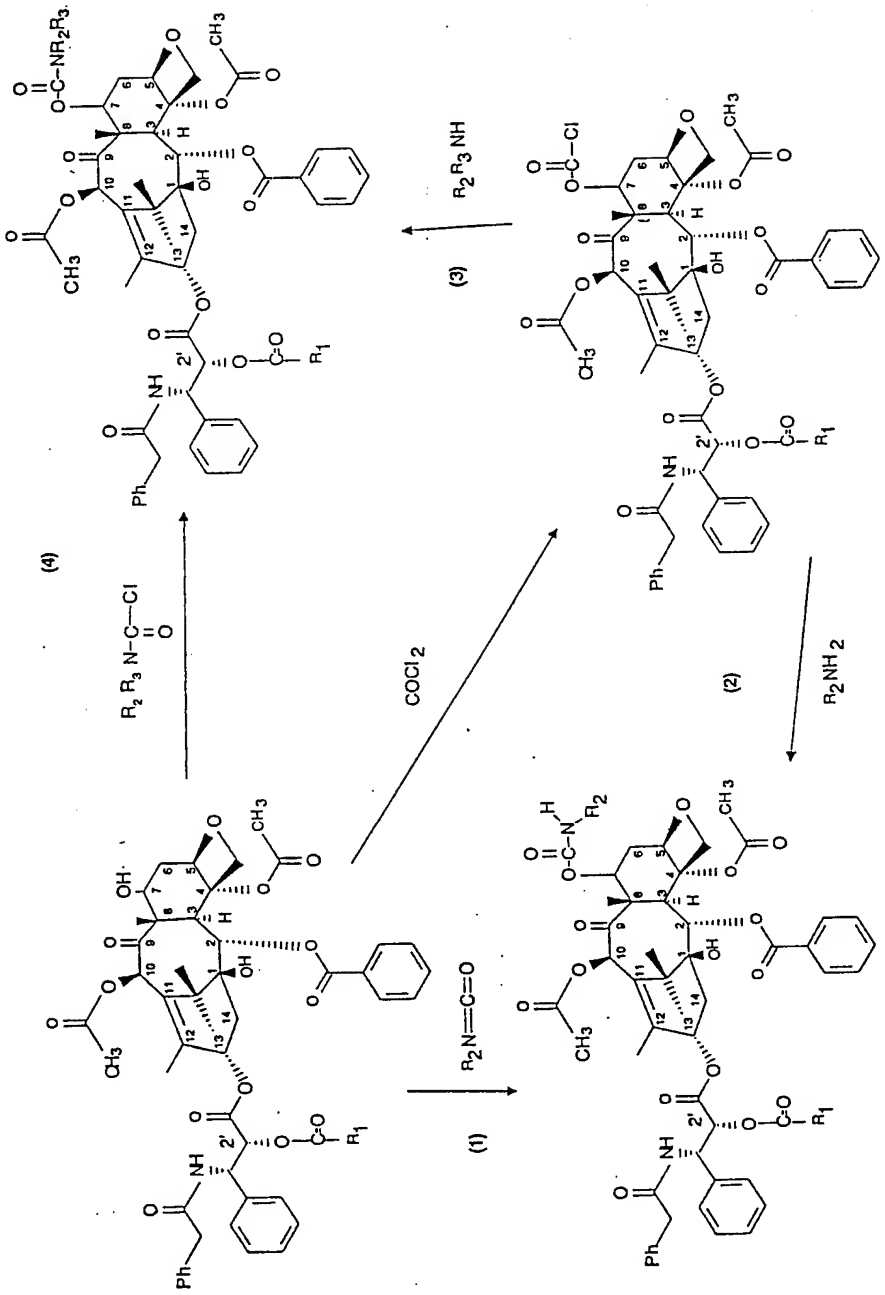
4) R_2R_3NCOCl where R_2 and R_3 are independently one of CH_3, alkyl, cycloalkyl, aryl, aralkyl or an α-substituted polyalkylene oxide derivative.

The conditions under which the anti-microtubule moiety and the activating reagent are reacted include:

the use of nonhydroxylic solvents such as anhydrous toluene, tetrahydrofuran, 1,2-dichloroethane, $CHCl_3$, CH_2Cl_2 or mixtures thereof;

carrying out the reaction at temperatures of from about 10-50 and preferably 25-35 degrees C;

allowing the reaction to proceed for times of up to two or more days. Several reaction schemes are set forth below.


(4)
$R_2R_3N-C-Cl$
O
(3)
R_2R_3NH
$COCl_2$
(2)
R_2NH_2
(1)
$R_2N=C=O$

Another aspect of the present invention provides methods of treatment for various medical conditions in mammals. The methods include administering an effective amount of a taxol-based conjugate as described herein to the mammal. The compositions are useful for, among other things, treating neoplastic disease, reducing tumor burden, preventing metastasis of neoplasms and preventing recurrences of tumor / neoplastic growths.

The amount of taxol-based conjugate used in the methods described herein may generally be described as that amount which effectively achieves the desired therapeutic result in mammals. Naturally, the dosages of the various taxol conjugates will vary somewhat depending upon the taxol-based moiety and the non-antigenic polymer selected for conjugation. In general, however, the conjugate may be administered in amounts ranging from about 5 to about 500 mg/m^2 per day, based the amount of the active taxol moiety in the conjugate. The range set forth above is illustrative and those skilled in the art will determine the optimal dosing of the conjugate selected based on clinical experience and the treatment indication.

The conjugates of the present invention can be included in one or more suitable pharmaceutical compositions for administration to mammals. The pharmaceutical compositions may be in the form of a solution, suspension, tablet, capsule or the like, prepared according to methods well known in the art. It is also contemplated that administration of such compositions may be by the oral and/or parenteral routes depending upon the needs of the artisan.

EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention.

EXAMPLE 1

PREPARATION of

2′ ACETYL 7-PEG 5000 CARBAMATE TAXOL USING mPEG-NCO

SCHEME 1

In this Example, mPEG-NCO was prepared in situ by placing 515 mg (0.010 mmol) of mPEG-NH_2 in a 100 ml round bottom flask and undergoing drying by azeotropic toluene distillation followed by conversion to mPEG-NCO as described in the parent application. The FTIR showed the isocyanate peak at 2263 cm^{-1}. The reaction mixture was cooled to room temperature before adding 60 mg of 2′-acetyltaxol prepared as described in Biochem. Biophys. R.S. Commun. 124, 329-336 (1984) and 10 mg Sn(II) octoate. The reaction was followed by HPLC on a C_8 reverse phase column with 3:1 methanol-H_2O as eluent. The reaction appeared to be complete when about 75% of 2′-acetyltaxol was converted to the corresponding PEG-derivative. The reaction product was evaporated to near dryness and precipitated with ether. Most of the unreacted 2′-acetyltaxol and any 2′, 7-diacetyltaxol present remained in the ether phase. The ether was decanted, and the precipitate was recrystallized from 20 ml of 2-propanol. The 7-carbamate-PEG derivative was isolated by centrifugation, washing with two 20 milliliter portions of 2-propanol, and finally drying under high vacuum to obtain 508 mg of product with less than 1% 2′-acetyltaxol and some non-functionalized PEG.

The FTIR spectrum of the purified compound had all the characteristic peaks of PEG in addition to peaks at 1748.6, 1741.2, 1726.5, 1663 cm^{-1} which are characteristic of the 2′-acetyltaxol molecule.

EXAMPLE 2

PREPARATION OF 7-GLYCINE CARBAMATE

ALTERNATE SCHEME 1

In this Example, 58 mg of 2′-acetyltaxol (0.067 mmol) was placed in 25 ml round-bottomed flask. Chloroform (ca 5.4 ml) was distilled directly over phosphorus pentoxide into this flask. Next, 138 mg of ethyl isocyanatoacetate (1.07 mmol) and dibutyltin dilaurate (300 mg) were added to the flask and the reaction mixture was heated to 40°C. The reaction was followed by HPLC on a C_8 reverse phase column with 3:1 methanol-water as the eluent. After 16 hours, the 2′-acetyltaxol peak disappeared completely and a new peak corresponding to the product appeared.

The mixture was concentrated to near dryness, precipitated with 25 ml of hexane, and then centrifuged. The supernatant contained only a very small amount of the desired product. The precipitate was dried under high vacuum and further purified by HPLC on a preparative C_8 column to yield 50 mg of product which was characterized by NMR. The product was subjected to hydrolysis by $NaHCO_3$ (20 mg) in 3:1 methanol-water (8 ml). After 30 minutes at room temperature, a new peak of lower retention time began to appear in the HPLC trace which corresponded to the hydrolysis of 2′-acetyl group (II). After 2 hours of further hydrolysis, a third peak appeared corresponding to the removal of the ethyl group of the side chain at 7 position (III). The reaction was

stopped when the ratio of II to III was about 40:60 because longer reaction times led to undesired hydrolysis of the taxane skeleton.

The product was isolated by extraction into methylene chloride. The mixture of II and III was separated from any impurities by HPLC.

EXAMPLE 3

PEGYLATION OF 2′-ACETYL TAXOL USING $MPEGNH_2$

SCHEME 2

2′-acetyltaxol (78mg, 89 μmol) was dissolved in 2 ml of dry 2-dichloroethane, and to this solution were added 13.2 mg of triphosgene (44.5 mmol) and 7.6 mg of pyridine (96.8 μmol) 7.6 mg. The reaction was followed by HPLC using the disappearance of 2′-acetyltaxol and appearance of a new peak with higher retention time. More triphosgene and pyridine were added until this conversion to chloroformate was greater than 80%. At this time one equivalent each of $mPEGNH_2$ and pyridine were added to complete the reaction. The resulting composition was found to have an HPLC peak which corresponded to the peak obtained for 2′ -acetyl-7-PEG urethane taxol prepared using mPEG-NCO described in Example 4 of the parent application herein. Confirmation of the compound was achieved by "spiking" with pure 2′-acetyl-7-PEG-(5000)-Carbamate taxol. The relative intensity of the HPLC peak increased, confirming that the product obtained by the two methods were the same.

EXAMPLE 4

SCHEME 3

In this Example, the procedure of Example 3 was repeated except that N-methyl-PEG-amine was employed instead of the $mPEGNH_2$ to yield the N-methyl-carbamate derivative of taxol.

EXAMPLE 5

Functionalization of 2′-Acetyltaxol with N-Methyl PEGamine. SCHEME 4

In this example, 100 ml of chloroform was added to 1 g (0.20 mmol) of $mPEGNCH_3.HCl$. The solution was dried by azeotropic distillation using molecular sieves placed in the side-arm of the Dean-Stark trap. After removal of 100 ml of chloroform, the reaction was cooled to room temperature and triphosgene (22 mg, 0.074 mmol), and triethylamine (42 mg, 0.42 mmol) were added, and the mixture was refluxed for 16 hours. An aliquot was withdrawn and checked by IR after removal of the solvent. The IR spectrum showed a strong peak at 1702 cm^{-1} indicating the formation of the carbamyl chloride. Next, 25 mg of 2′-acetyltaxol(0.029 mmol) followed by 0.02 ml of triethylamine was added to the flask and refluxed. After 16 hours, 6% conversion occurred. Additional chlorocarbamate (1 g) prepared separately as described above was added and reflux continued for 40 hours. A 10% yield of the desired product was obtained. The retention time of this peak on the C_8 column was similar to that of 2′-acetyltaxol PEGylated using MPEGNCO indicating that the desired conjugation had occurred.

EXAMPLE 6

In this Example, the activity of several taxol compositions against various malignant human cell lines was compared. Compositions A, B and C were dissolved in DMSO prior to dilution for cell testing. The inhibitory concentrations (IC_{50}) were determined using standard procedures and after 72 hours, the results were reported. All cell lines are obtained from the ATCC.

A549 = HUMAN LUNG CARCINOMA
BT20 = HUMAN BREAST CARCINOMA
C4i = HUMAN CERVICAL CARCINOMA
A375 = HUMAN MALIGNANT MELANOMA

IC_{50} MICROMOLAR QUANTITIES OF TAXOL DERIVATIVES

TABLE I

	CELL LINE			
COMPOSITION	A549	BT20	C4i	A375
A. TAXOL	0.01	0.01	0.01	0.002
B. 2′-ACETYL TAXOL	0.01	----	0.014	----
C. 7-ACETYL TAXOL	0.05	----	0.06	0.003
D. 7-GLYCINE CARBAMATE* TAXOL	0.33	0.42	0.30	0.13
E. 2′-ACETYL-7-PEG(5000) CARBAMATE TAXOL	4.38	2.00	3.19	6.86
F. 2′-OH-7-PEG(5000) CARBAMATE TAXOL	6.49	2.66	2.12	1.00

* as a 60:40 mixture of ester to acid

It can be seen from the above table, the 7-carbamates showed significant activity against these cell lines. Even though samples D, E and F were less active than the non-carbamate taxols, these compositions nonetheless have significant utility as therapeutic compounds for a variety of neoplastic diseases.

EXAMPLE 7

In this Example, the same taxol derivatives A-F described above in Example 6 were evaluated in two more malignant cell lines. Cell line P388/0 is a doxorubicin-sensitive mouse lymphoid neoplasm; P388/ADR is a doxorubicin-resistant mouse lymphoid neoplasm. Both were obtained from the Southern Research Institute, Birmingham, AL. The results are set forth below in Table II.

TABLE II

COMPARATIVE ACTIVITY OF PEG-TAXOL

IC_{50} MICROMOLAR QUANTITIES OF TAXOL DERIVATIVES

		P388/0	P388/ADR
A.	TAXOL	0.07	0.32
B.	2'-ACETYL TAXOL	0.13	0.60
C.	7-ACETYL TAXOL	0.04	0.38
D.	7-GLYCINE CARBAMATE* TAXOL	0.60	0.80
E.	2'-ACETYL-7-PEG(5000) CARBAMATE TAXOL	10.6	10.6
F.	2'-OH-7-PEG(5000) CARBAMATE TAXOL	4.0	8.5

* as a 60:40 mixture of ester to acid

Referring now to Table II, it can be seen that the compositions maintained activity against these cell lines in spite of the substantial 7 position modifications. What is especially noteworthy is that Sample E was essentially as active against the resistant strain as it was against the non-resistant line. The 2'-OH derivative also showed substantial retained activity against this resistant cell line. While applicants are not bound by

theory, it is believed that the PEG modification may overcome certain tumor cell resistance in a mode of action separate from the composition's prolonged in vivo disappearance time.

EXAMPLE 8

In this Example, the water solubility of the taxol carbamates prepared in accordance with the present invention is demonstrated. Each of the derivatives tested demonstrated superior water solubility when compared to unmodified molecule. Indeed, the 7-carbamate-PEG taxol was over 600 times more soluble in water than the unmodified material.

TABLE III

WATER SOLUBILITY COMPARISON

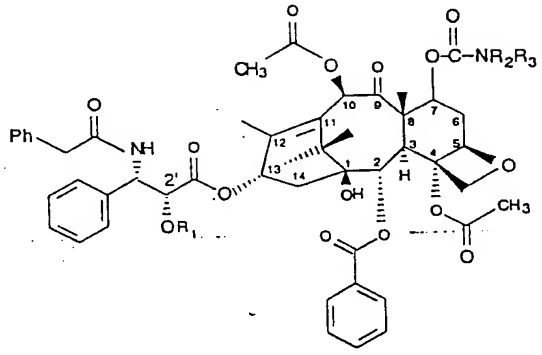
COMPOSITION	AQUEOUS SOLUBILITY
Taxol	0.5 mg/ml
7-PEG(5000)Carbamate Taxol	> 300.0 mg/ml
7-Glycine-Carbamate Taxol	~1.0mg/ml

While there have been described what are presently believed to be the preferred embodiments of the invention, those skilled in the art will realize that changes in modification may be made there to without departing from the spirit of the invention. It is intended to claim all such changes and modifications as all within the true scope of the invention.

WHAT IS CLAIMED IS:

1. A composition of matter comprising the structure:

(I)



wherein:
R_1 = H or -CO-X, wherein X = alkyl or aryl;
R_2 = one of H, CH_3, alkyl, cycloalkyl, aryl, aralkyl, aryl$(CH_2)_nY$ or $(CH_2)_nY$, wherein n = 1-12 and Y = OH, NH_2, CHO or $COOR_4$ where R_4 = alkyl, substituted alkyl, phenyl or substituted phenyl, or an α-substituted polyalkylene oxide derivative; and
R_3 = one of H, CH_3, alkyl, cycloalkyl, aryl, aralkyl or an α-substituted polyalkylene oxide derivative;
except that at least one of R_1, R_2 and R_3 is an α-substituted polyalkylene oxide derivative.

2. The composition of claim 1, wherein R_1 = an acetyl.

3. The composition of Claim 2, wherein said α-substituted polyalkylene oxide derivative is a polyethylene oxide derivative.

4. The composition of Claim 1, wherein said α-substituted polyalkylene oxide derivative is an α-alkyl-

polyethylene oxide.

5. The composition of Claim 4, wherein said α-alkyl-poly-ethylene oxide is methyl(polyethylene oxide).

6. The composition of Claim 1, wherein said α-substituted polyalkylene oxide derivative has a molecular weight of from about 200 to about 10,000.

7. The composition of Claim 6, wherein said α-substituted polyalkylene oxide derivative has a molecular weight of from about 2000 to about 7500.

8. The composition of Claim 7, wherein said α-substituted polyalkylene oxide derivative has a molecular weight of about 5000.

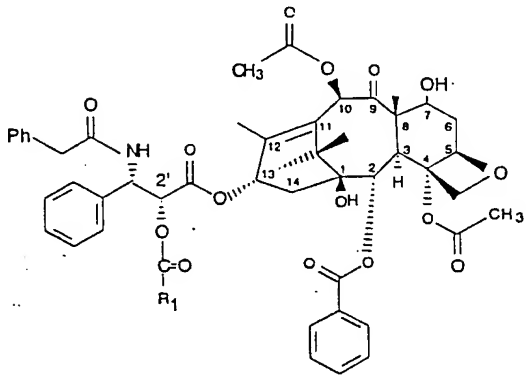
9. The composition of Claim 1 wherein at least one of said R_2 and R_3 are α-substituted polyalkylene oxide derivatives.

10. The composition of Claim 9, wherein at least both of said R_2 and R_3 are α-substituted polyalkylene oxide derivatives .

11. The composition of Claim 3, wherein said α-substituted polyalkylene oxide derivative is selected from the group consisting of polyalkylene oxide homopolymers, polyalkylene oxides, copolymers of polyoxyethylenated polyols and block copolymers of polyalkylene oxides.

12. A method of preparing an anti-microtubule composition, a moiety having the structure II comprising reacting

(II)



wherein R_1 = alkyl or aryl;
with an activating reagent under conditions sufficient to effect modification of said moiety by said reagent while maintaining at least a portion of the anti-microtubule activity of said moiety.

13. The method of Claim 12, wherein said activating reagent is R_2NCO where R_2 is one of CH_3, alkyl, cycloalkyl, aryl, aralkyl, aryl$(CH_2)_nY$ or $(CH_2)_nY$, wherein n = 1-12 and Y = OH, NH_2, CHO or $COOR_4$ where R_4 = alkyl, substituted alkyl, phenyl or substituted phenyl, or an α-substituted polyalkylene oxide derivative or an α-substituted polyalkylene oxide derivative.

14. The method of claim 12, wherein said activating reagent is R_2R_3NCOCl and R_2 and R_3 are independently one of CH_3, alkyl, cycloalkyl, aryl, aralkyl or an α-substituted polyalkylene oxide derivative.

15. The method of claim 12, wherein said activating reagent is R_2NH_2 and R_2 is one of H, CH_3, alkyl, cycloalkyl, aryl, aralkyl or an α-substituted polyalkylene oxide derivative.

16. The method of claim 12, wherein said activating reagent is R_2R_3NH and R_2 and R_3 are independently one of CH_3, alkyl, cycloalkyl, aryl, aralkyl or an α-substituted polyalkylene oxide derivative.

17. The method of claim 12, wherein said conditions include dissolving said composition (II) in a non-hydroxylic solvent prior to reacting with said reagent.

18. The method of claim 17, wherein said non-hydroxylic solvent is selected from the group consisting of tetrahydrofuran, 1,2-dichloroethane, toluene, $CHCl_3$, CH_2Cl_2 and mixtures thereof.

19. The method of claim 12, wherein said conditions include reacting said composition (II) and said reagent in a substantially anhydrous environment.

20. The method of claim 12, wherein said reacting is carried out at a temperature of from about 10 to about 60 degrees C.

21. The method of claim 20, wherein said reacting is carried out at a temperature of from about 20 to about 45 degrees C.

22. The method of claim 21, wherein said reacting is carried out at a temperature of from about 25 to about 40 degrees C.

23. The composition prepared in accordance with the method of claim 12.

24. A method of treating neoplastic disease in mammals, comprising administering to a mammal in need of such treatment an effective amount the composition of Claim 1.

25. A biologically active conjugate comprising a moiety having anti-microtubule activity in mammals linked by any atom to a substantially non-antigenic substance.

26. The conjugate of Claim 25, wherein said moiety is taxol.

27. The conjugate of Claim 25, wherein said moiety is a vinca alkaloid.

28. The conjugate of Claim 25, wherein said non-antigenic substance comprises polyethylene glycol.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/02441

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :A61K 31/335; C07D 305/14; C12N 9/96
US CL :514/449, 549/510, 511; 435/188
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/449, 549/510, 511

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,534,899 (SEARS) 13 AUGUST 1985, see entire document.	1-11, 23-28
Y	US, A, 4,942,184 (HAUGWITZ ET AL) 17 JULY 1990, see entire document.	12-22
Y	US, A, 5,059,699 (KINGSTON ET AL) 22 OCTOBER 1991, see entire document.	1-11, 23-28
Y	EUROPEAN POLYMER JOURNAL, Volume 19, No. 12, issued 1983, Zalipsky et al, "Attachment of Drugs to Polyethylene Glycols", See pages 1177-1183.	1-11, 23, 25-28
Y	POLYMER PREPRINTS, Vol. 31, issued 1990, Nathan et al., "Polyethylene Glycol-Lysine Copolymers: New Biocompatible Polymers for Biomedical Applications", See pages 213-214.	1-11, 23, 25-28

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
10 JUNE 1994

Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/02441

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF MEDICINAL CHEMISTRY, Vol. 16, No. 5, issued 1973, Weiner et al. "Polyethylene Glycol Derivatives of Procaine", see pages 573-574.	1-11, 23, 25-28
A	TETRAHEDRON LETTERS, No. 31, issued 1974. Brandstetter et al. "Neue Polymer-Schutzgruppe In Der Oligonucleolidsynthese 2-hydroxy-athylphenylthioather Von Polyathylenglycol", pages 2705-2708.	1-28